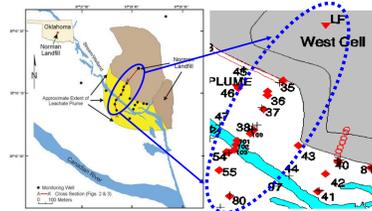


## ABSTRACT

The Norman Landfill is a closed municipal solid waste landfill located on an alluvium associated with the Canadian River in Norman, Oklahoma. It has operated as a research site since 1994 because it is typical of many closed landfill sites across the U.S. Leachate from the unlined landfill forms a groundwater plume that extends downgradient approximately 250 m from the landfill toward the Canadian River. To investigate the impact of the landfill leachate on the diversity and functional structure of microbial communities, groundwater samples were taken from eight monitoring wells at a depth of 5m, and analyzed using a comprehensive functional gene array covering about 50,000 genes involved in key microbial processes, such as biogeochemical cycling of C, N, P, and S, and bioremediation of organic contaminants and metals. Wells are located within a transect along a presumed flowpath with different distances to the center of the leachate plume. Our analyses showed that microbial communities were obviously impacted by the leachate-component from the landfill. The number of genes detected and microbial diversity indices in the center (LF2B) and its closest (MLS35) wells were significantly less than those detected in other more downgradient wells, while no significant changes were observed in the relative abundance (i.e., percentage of each gene category) for most gene categories. However, the microbial community composition or structure of the landfill groundwater did not clearly show a significant correlation with the distance from well LF2B. *Burkholderia* sp. and *Pseudomonas* sp. were found to be the dominant microbial populations detected in all wells, while *Bradyrhizobium* sp. and *Ralstonia* sp. were dominant populations for seven wells except LF2B. In addition, Mantel test and canonical correspondence analysis (CCA) indicate that pH, sulfate, ammonia nitrogen and dissolved organic carbon (DOC) have significant effects on the microbial community structure. The results suggest that the leachate from unlined landfills significantly impact the structures of groundwater microbial communities, and that more distal wells recover by natural attenuation.

## SITE DESCRIPTION

Norman Landfill is a closed municipal solid waste landfill sited on the Canadian River alluvial aquifer in Norman, Oklahoma. The landfill, which is unlined and has no leachate collection system, received solid waste for surface disposal beginning in 1922 and disposal in trenches during 1960–85. It has been designated as a U.S. Geological Survey research site under the USGS Toxic Substances Hydrology Program since 1995 (<http://ok.water.usgs.gov/projects/norlan/>).



## METHODS

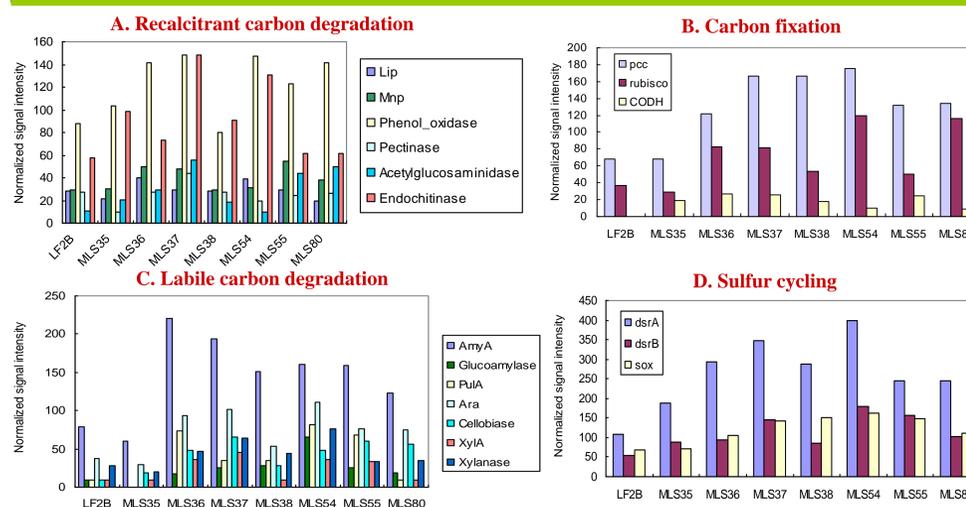
- DNA extraction, amplification and labeling:** Eight wells at the depth of 5m were sampled: LF2B, MLS35, MLS36, MLS37, MLS38, MLS54, MLS55, and MLS80. 5L underground water for each well was filtered through 0.45µm pore size membrane on-site and immediately chilled and sent to the laboratory for later analysis. High molecular weight DNA was extracted by freeze-grinding methods. 100ng DNA was amplified using a TempliPhi kit, and the amplification products were labeled with Cy-5 using random priming method.
- GeoChip hybridization, scanning and image analysis:** A functional gene array (GeoChip 3.0) was used for underground water DNA hybridization. All hybridizations were carried out in triplicate at 45°C for 10 hours with 50% formamide using a TECAN HS4800. The array was scanned by a ScanArray Express Microarray Scanner at 633 nm. ImaGene version 6.0 was then used for image quantification.
- GeoChip data analysis:** Functional gene diversity was calculated using Simpson's 1/D, 1-D, Shannon Weaver's H' and evenness.
- Statistical analysis:** Detrended correspondence analysis (DCA) was used to determine the overall functional changes. Multivariate statistical analyses including ANOSIM, Mantel test, CCA and partial CCA analyses were performed to link microbial communities to environmental variables.

## CHANGES IN FUNCTIONAL GENE DIVERSITY AND RELATIVE ABUNDANCE

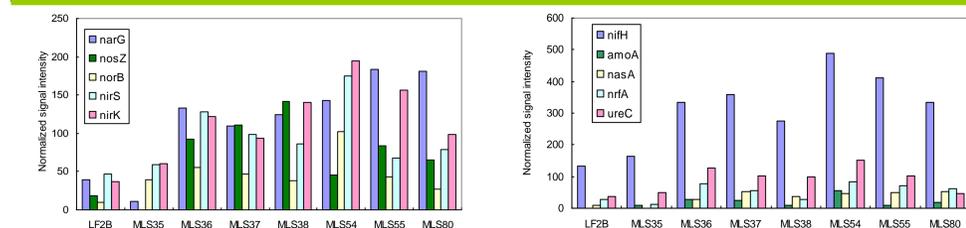
Table 1. Gene overlap (italicized), uniqueness (bold) and diversity indices of Landfill samples.

Sample name	LF2B	MLS35	MLS36	MLS37	MLS38	MLS54	MLS55	MLS80
LF2B	<b>36(7.86%)</b>	249(31.80%)	339(24.55%)	363(26.40%)	299(24.53%)	349(20.17%)	319(25.34%)	287(24.53%)
MLS35		<b>70(12.20%)</b>	387(26.71%)	395(27.07%)	311(23.51%)	416(23.38%)	343(25.39%)	324(25.94%)
MLS36			<b>201(15.93%)</b>	710(38.76%)	509(28.08%)	790(37.74%)	582(32.33%)	525(30.24%)
MLS37				<b>235(18.36%)</b>	547(30.51%)	764(35.75%)	582(32.01%)	536(30.75%)
MLS38					<b>298(28.11%)</b>	546(25.57%)	479(28.16%)	413(25.09%)
MLS54						<b>426(26.28%)</b>	623(29.41%)	561(27.25%)
MLS55							<b>265(23.66%)</b>	471(28.58%)
MLS80								<b>229(22.92%)</b>
Diversity indices								
Shannon-weaver H'	5.88	6.12	6.89	6.90	6.66	7.11	6.74	6.62
Simpson's (1/D)	266.26	344.97	710.81	714.72	510.97	884.61	555.82	537.85
SimpsonE	0.58	0.60	0.56	0.56	0.48	0.55	0.50	0.54
<b>Num. of detected genes</b>	<b>458</b>	<b>574</b>	<b>1262</b>	<b>1280</b>	<b>1060</b>	<b>1621</b>	<b>1120</b>	<b>999</b>

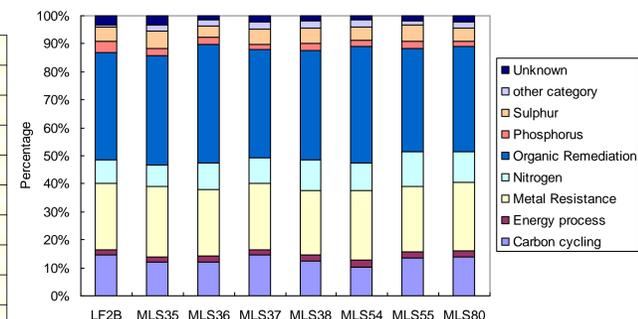
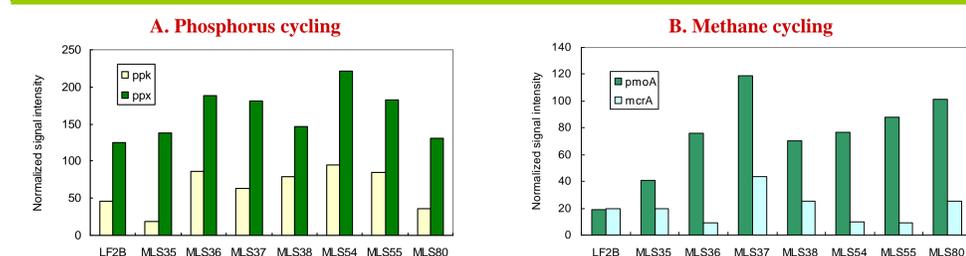
## GENES INVOLVED IN CARBON AND SULFUR CYCLING



## GENES INVOLVED IN NITROGEN CYCLING

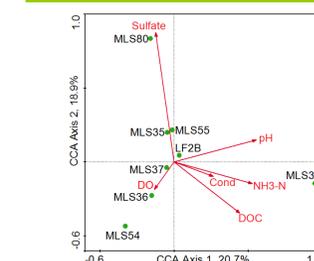


## GENES INVOLVED IN PHOSPHORUS AND METHANE CYCLING

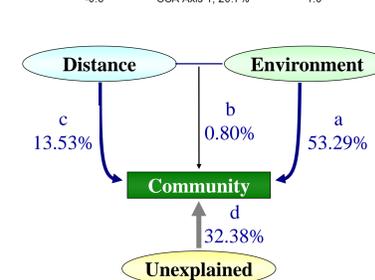


The microbial diversity was found to be the lowest in LF2B, while highest in MLS54. All the wells have higher percentages of organic remediation genes at 36.78%-42.39% and metal resistance genes at 23.11%-25.09%. The relative abundances of the gene categories were similar.

## RELATIONSHIPS BETWEEN COMMUNITY STRUCTURE AND ENVIRONMENTAL VARIABLES



Canonical correspondence analysis (CCA) of GeoChip hybridization signal intensities and groundwater environmental variables. Environmental variables were chosen based on significance calculated from individual CCA results and VIF calculated during CCA. The percent of variation explained by each axis is shown, and the relationship is significant ( $p=0.022$ ).



Variation partitioning analysis (VPA) was performed to better understand how much each environmental variable influenced the functional community structure. Environmental variables and distance showed a significant correlation ( $p=0.034$ ) with community composition. Distance was able to independently explain 13.53% of the variation observed while environmental variables explained 53.29%. About 32.38% of the variation was unexplained.

## SUMMARY

- ✓ The microbial community structure within the leachate plume showed high heterogeneity from each well and clearly impacted by landfill leachate.
- ✓ The leachate caused a decrease in microbial diversity, but recovery with distance was also noted.
- ✓ Environmental variables greatly influenced functional gene composition.

## ACKNOWLEDGEMENT

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